

Entomocidal Effect of Entomopathogenic Fungus *Beauveria bassiana* and New Chemistry Insecticides against *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera) Under Controlled Conditions

Muhammad Ismail¹, Waqas Wakil¹, Nawaz Haider Bashir^{2*}, Waheed ul Hassan³ and Usman Wali Muhammad¹

¹ Department of Entomology, University of Agriculture Faisalabad, Pakistan.

² College of Plant Protection, Yunnan Agriculture University, Kunming, Yunnan 650201, China.

³ Department of Plant Protection, Bogor Agriculture University, Indonesia.

*Corresponding author email id: nawazuca@gmail.com

Abstract – *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera) is one of the notorious and polyphagous insect pest with broad host range of more than one hundred plants including 40 plant families. This pest flourishes under favorable conditions causing 70 to 100% yield losses to crops at their blossoming and vegetative stages. Many species of insect pests have significantly developed resistance to different group of chemical insecticides. Subsequently controlling insect pests by the usage of synthetic chemicals is expensive and ecologically harmful, so there has been a developing hunt for naturally arising with integration, economical and more globally safe means. Presently *B. bassiana* (1.3×10^6 spores/ml) and three new chemistry insecticides [emamectin benzoate (Timer[®]), flubendiamide (Belt[®]) and chlorantraniliprole (Coragen[®])] alone and in mixtures at dose less than the 2 folds of recommended field dose were tested against 2nd and 4th instar larvae of *S. litura*, under laboratory conditions using larval immersion and leaf dip method. Interaction of *B. bassiana* and new chemistries showed the higher percentage of mortalities in both tested instars larvae as compared to alone treatment. Bb* Coragen[®] (91.43%, 86.14%) interaction showed the highest larval mortality followed by Bb*Belt[®] (84.60%, 76.88%) and Bb*Timer[®] (74.60%, 70.86%) for 2nd and 4th instar larvae respectively. Similarly decrease in pupation rate and adult emergence was observed in alone and combined treated applications. *B. bassiana* and sub-lethal doses of new chemistries significantly increase the larval and pupal duration while decrease in adult longevity was observed.

Keywords – Beauveria Bassiana, Emamectin Benzoate, New Chemistry Insecticides, Spodoptera Litura.

I. INTRODUCTION

Insect pests cause enormous damage to crops. Cross resistance to numerous insecticides is worldwide problem and even most effective chemical insecticides have failed to control *S. litura*. In many countries including Pakistan, high levels of cross resistance to synthetic insecticides have caused sporadic outbreaks of this voracious pest and enormous damage to our crops [1]. To overcome the resistance problem to insecticides enforced farmers to use higher dosages, increased number of applications and sometimes the mixtures of insecticides which not only adversely effects the natural enemies but also increase their cost of production and environmental hazards to ecosystem [2].

Emamectin benzoate (Timer[®]), a semi synthetic bio insecticide is a macro cyclic lactone derived from a naturally occurring compound, avermectin which is extracted from the fermentation of actinomycetes *Streptomyces avermitilis* [3]. Avermectins acts on glutamate and GABA sites in the insect chloride channels. Chloride ion flux is produced through opening of channel to neuron cells results in disruption of nerve impulse and loss of cell functions which causes the cessation of feeding, paralyzed irreversibly and ultimately death of insects [4]. Chlorantraniliprole (Coragen[®]) is the new class of chemistry, the anthranilicdiamides which exhibit their action through activation of ryanodine receptors [5]. Anthranilicdiamide possesses favorable environmental and toxicological properties due to their low residual effect and low mammalian toxicity. It exhibits stomach, contact, ovicidal and ovi-larvicidal activity [6]. Ca²⁺ signaling plays a vital role in many biological processes, including neurotransmitter release and muscle contraction [7] which is controlled by balancing the Ca²⁺ levels in the muscle cells through voltage-gated channels and ryanodine receptors (RyRs) [8,9]. The chlorantraniliprole acts on the ryanodine receptors, makes it open and resultantly release of all the stored calcium, which causes the cessation of feeding, muscle paralysis and ultimately death of insects [10]. Flubendiamide (Belt[®]) is the new class of chemistry, the phthalic acid diamides discovered by Nihon Nohyaku in 1998. It is most effective against lepidopterous larvae and adults having low residual effect and low mammalian toxicity. It has the same mode of action as the chlorantraniliprole disturbing the calcium channel in the muscle by fixing it at ryanodine receptors (RyR) [11].

The Approach of joint application of two pest controlling agents is based on idea that the myco pathogen makes the host to become more susceptible to chemical insecticides and in response insecticides weaken the pest sufficiently to make it more vulnerable to disease [12]. Studies revealed that all the life stages of *S. litura* were not equally vulnerable to infection by entomopatho fungi [13]. The objective of the current investigation was to study the effect of *B. bassiana* and three new chemistries and their combined effect on mortality and some biological parameters of *S. litura* under laboratory conditions.

II. MATERIALS AND METHODS

1.1. Insect Rearing

Larval population of *S. litura* was collected from different tomato fields of AARI, Faisalabad and University of Agriculture, Faisalabad. Larvae were reared on chickpea-based artificial diet [14] in the IPM laboratory, Department of Entomology, University of Agriculture, Faisalabad.

1.2. Fungal Isolation and Conidia Preparation

Isolates of *B. bassiana* were obtained from laboratory collection of IPM Microbial Laboratory, Department of Entomology, UAF. These spores from that isolate were inoculated to media plates containing Potato Dextrose Agar (PDA) and these petri plates were incubated at $28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ R.H. to get further growth of fungi. The serial dilutions of the fungal culture were prepared and fungal concentration 1.3×10^6 conidia/ml was calculated using haemocytometer.

1.3. Test Chemicals

The commercial formulations of the following chemicals were used:

- I. The emamectin benzoate; Timer[®] (Ali Akbar Group) 1.9% EC.
- II. The Flubendiamide; Belt[®] (Bayer Crop Science) 48% SC.
- III. The chlorantraniliprole; Coragen[®] (Du Pont) 20% SC.

1.4. Bioassay

- Eight treatments were used including application of *B. bassiana* (1.3×10^6) and three insecticides alone and in combination of *B. bassiana* with control.
- Fifteen larvae were immersed into a conidial suspension of *B. bassiana* for 10s and allowed to dry for 10 minutes in a sterile petri plate of 3cm diameter [15].
- Leaf dip method was used for application of synthetic insecticides, different insecticides were placed in 5 cm diameter petri plates and ten 2nd instar larvae were allowed to feed on it [16].
- To check the combined effect of *B. bassiana* and other three insecticides, the larvae firstly were dipped in *B. bassiana* spores suspension for 10s and dried for 10 minutes. After this, these larvae were put into 5 cm petri plates containing leaves dipped in insecticides.
- The data for larval development, larval mortality, pupal development, pupation percentage and rate of adult emergence were recorded.

Conducting experiment for sporulation and mycosis the dead cadavers in each bioassay were taken and placed on a sterile petri dish and refrigerated at $4-5^\circ\text{C}$. Then dead cadavers were surface sterilized by dipping in 0.05% sodium hypochlorite and followed by 2-3 washing with deionized water. After 9-10 days, emerging whitish conidial spore from the body of cadavers was examined by using compound microscope. Spore production in dead insects was determined after 15 days of incubation. Mycosed insects in each treatment were thoroughly mixed in 10ml distilled water containing 0.01% Tween 80 and stirred for 10 minutes. The numbers of conidia/ml were counted with the help of haemocytometer by using compound microscope

[17].

1.5. Statistical Analysis

Mortality data was corrected by using Abbott formula [18]. The data was analyzed by using Minitab software.

III. RESULTS

3.1. Larval Mortality of 2nd Instar

Second instar larvae of *S. litura* dipped in conidial concentration of *B. bassiana* as well fed on diet containing lower doses of new chemistries [chlorantraniliprole (25ppm), flubendiamide (24ppm) and emamectin benzoate (9.5ppm)], the interaction showed significantly difference from their alone concentration for larval mortality, pupation and adult emergence. (Mortality: $F_{[6, 56]} = 16.4$; $p < 0.001$; Pupation: $F_{[6, 56]} = 22.8$; $p < 0.001$; adult emergence: $F_{[6, 56]} = 26.6$; $p < 0.001$ S). All new chemistries and *B. bassiana* combination showed significantly higher mortalities than their alone dose rate. The combination of *B. bassiana* (1.3×10^6 conidia/ml) with low dose of chlorantraniliprole showed highest mortality 48.68% after day 2 and after day 10 it was increased to 91.43% followed by Bb* flubendiamide (84.60%) and Bb*emamectin benzoate (74.60%) interaction. Lower mortalities were recorded when Bb and insecticides were applied alone. Emamectin benzoate showed highest mortality (68.57%) alone but still lower than Bb*chemical interaction followed by chlorantraniliprole (64.29%), flubendiamide (58.25%) and *B. bassiana* (53.70%). Similarly highest pupation and adult emergence was observed in Bb alone 33.33% and 28.89% respectively. In case of Bb*chlorantraniliprole interaction no pupation and adult emergence was recorded (Table 4.1, Fig. 4.1).

3.2. Larval Mortality of 4th Instar

When 4th instar larvae of *S. litura* dipped in conidial concentration of *B. bassiana* were fed on diet containing lower doses of new chemistries [chlorantraniliprole (25ppm), flubendiamide (24ppm) and emamectin benzoate (9.5ppm)], the interaction showed significantly difference from their alone concentration for larval mortality, pupation and adult emergence. (Mortality: $F_{[6, 56]} = 18.6$; $p < 0.001$; Pupation: $F_{[6, 56]} = 28.2$; $p < 0.001$; adult emergence: $F_{[6, 56]} = 32.4$; $p < 0.001$ S). All new chemistries and *B. bassiana* combinations showed significantly higher mortalities than their alone dose rate. The combination of *B. bassiana* (1.3×10^6 conidia/ml) with low dose of chlorantraniliprole showed highest mortality 45.82% after day 2 and after day 10 it was increased to 86.14% followed by Bb*flubendiamide (76.88%) and Bb*emamectin benzoate (70.80%) interaction. Lower mortalities were recorded when Bb and insecticides were applied alone. Emamectin benzoate showed highest mortality (62.33%) alone but still lower than Bb*chemical interaction followed by chlorantraniliprole (58.47%) flubendiamide (53.76%) and *B. bassiana* (45.40%). Similarly lowest pupation and adult emergence was observed in Bb*chlorantraniliprole interaction 4.44% and 0.00% respectively. While the highest pupation and adult emergence was recorded 42.22% and 34.82% respectively as Bb applied alone (Table 4.2, Fig. 4.2).

3.3. Mycosis and Sporulation in 2nd Instar of *S. Litura*

Mycosis and sporulation was recorded maximum (83.94%, 7.8×10^5 conidia/ml) in second instar larvae of *S. litura* when Bb applied alone, while least mycosis and sporulation was observed (24.12%, 3.4×10^5 conidia/ml) in Bb* emamectin benzoate interaction (Fig. 4.3, 4.4).

3.4 Mycosis and Sporulation in 4th Instar of *S. Litura*

Mycosis and sporulation was recorded maximum (81.80%, 6.9×10^5 conidia/ml) in 4th instar larvae of *S. litura* when Bb applied alone, while least mycosis and sporulation was observed (21.24%, 3.1×10^5 conidia/ml) in Bb* emamectin benzoate interaction (Fig. 4.3, 4.4).

3.5. Developmental Duration of *S. Litura*

3.5.1. Larval Duration

Significant difference in larval duration of treated and untreated population of *S. litura* was observed. Maximum larval duration of 24.78 days was recorded in Bb* chlorantraniliprole treated population followed by Bb* flubendiamide (23.22 days) and Bb* emamectin benzoate (21.89 days) interaction as compared to untreated 16.89 days for larval development. Similarly in case of alone treatment, maximum larval duration of 21.11 days was recorded in emamectin benzoate treated population followed by chlorantraniliprole (20.56 days), flubendiamide (19.56 days) and *B. bassiana* (17.89 days) respectively (Table 4.3).

3.5.2. Pupal Duration

Maximum pupal duration of 13.56 days was recorded in Bb*chlorantraniliprole treated population followed by Bb* flubendiamide (12.56 days) and Bb*emamectin benzoate (11.89 days) interaction as compared to untreated 8.56 days for pupal development. Similarly in case of alone treatment, maximum pupal duration of 11.11 days was recorded in emamectin benzoate treated population followed by chlorantraniliprole (10.89 days), flubendiamide (10.11 days) and *B. bassiana* (9.22 days) respectively (Table 4.3).

3.5.3. Adult Longevity

Minimum adult span 5.67 days was recorded in Bb*chlorantraniliprole treated population followed by Bb*flubendiamide (6.67 days) and Bb*emamectin benzoate (7.33 days) interaction as compared to untreated 10.33 days of adult longevity. Similarly in case of alone treatment, minimum adult duration of 7.78 days was recorded in emamectin benzoate treated population followed by chlorantraniliprole (8.56 days), flubendiamide (9.22 days) and *B. bassiana* (9.78 days) respectively (Table 4.3).

IV. DISCUSSION

Laboratory results proved that synthetic insecticides has caused the physiological changes in insects which make more susceptible to hyphomycetes, resultantly fungal spores easily penetrate into insect cuticle and produced extensively vegetative growth in haemolymph which deplete the nutrition and ultimately cause the death of host, hypothetical proposed by many scientists [3-7].

Efficacy of entomopathogenic fungi, *B. bassiana*, *M. anisopliae*, *Lecanicillium sp.* and *P. fumosoroseus* against *S. litura* has been described by many researchers [8-10].

There are many examples where virulence of entomopathogenic fungi had been increased by using them with selective insecticides against targeted insect pests [11, 12]. The combined effect of *M. anisopliae* and *B. bassiana* with selective insecticides were founded to be more effective than the alone concentration of entomofungus or chemical insecticides against *Helicoverpa armigera* larvae [13].

Results of our study regarding efficacy of *B. bassiana* and new chemistries similar to the findings of [13, 29, 30] and [31] that *B. bassiana* was highly virulent to *Spodoptera sp.* Older larvae were founded less vulnerable to fungal infection than the younger larvae [14, 15] and susceptibility of entomofungus to insects decreases with the increase in age of larvae of the targeted host [24, 34, 35]. Similarly efficacy of new chemistries against *S. litura* reported by many scientists [36, 41] showing the minimum LC₅₀ values against *S. litura* which corresponds to our results of larval mortality at lower dosages. Consequently the combined effect of *B. bassiana* and new chemistries enhance the virulence of entomopathogenic fungi corresponds to results of [11, 16]. A resembled array of retarded development of several lepidopterous insects [43, 44] is confirming that the present findings of slower growth in *S. litura* when treated with *B. bassiana*, chlorantraniliprole, flubendiamide, emamectin benzoate alone and in combination.

Sporulation and mycosis is an important tool of disease proliferation from dead cadavers to healthy insect population and should be given an importance in fungal biocontrol agent's selection [7]. Sporulation and mycosis play important role in spreading and multiplication of *B. bassiana* from cadavers to healthy one [17]. In our experiment alone concentration of *B. bassiana* produces more sporulation and mycosis in dead larvae of 2nd and 4th instars of *S. litura*. The equally increase in sporulation and mycosis from dead cadavers of *Chiloptartellus* and *Rhyzopertha dominica* at low dose rate of *B. bassiana* were observed by [18, 19].

V. CONCLUSION

Results indicate that the combination of *B. bassiana* and new chemistry insecticides at low dosage could be developed as potential control agent against economically important Insect pest *S. litura*. However mortality which was obtained in laboratory experiment did not predict the field mortality, so field trials were conducted to check the combined efficacy of *B. bassiana* and *Bacillus thuringiensis (Bt)* as a potential pest management tool.

REFERENCES

- [1] M.A. Saleem, M. Ahmad, M. Ahmad, M. Aslam, and A. H. Sayyed, "Resistance to selected organochlorin, organophosphate, carbamate and pyrethroid, in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan," Journal of economic entomology, vol. 101, 2008, pp. 1667-1675.
- [2] S. S. Sharma, K. Gill, M. S. Malik, and O. P. Malik, "Insecticidal, antifeedant and growth inhibitory activities of essential oils of some medicinal plants," Insecticidal, antifeedant and growth inhibitory activities of essential oils of some medicinal plants., vol. 22, 2000, pp. 373-377.

- [3] R. W. Burg, B. M. Miller, E. E. Baker, J. Birnbaum, S. A. Currie, R. Hartman, et al., "Avermectins, new family of potent anthelmintic agents: producing organism and fermentation," *Antimicrobial agents and Chemotherapy*, vol. 15, 1979, pp. 361-367.
- [4] S. P. Rohrer and J. P. Arena, "Ivermectin interactions with invertebrate ion channels," ed: ACS Publications, 1995.
- [5] M. Fill and J. A. Copello, "Ryanodine receptor calcium release channels," *Physiological reviews*, vol. 82, 2002, pp. 893-922.
- [6] G. P. Lahm, T. M. Stevenson, T. P. Selby, J. H. Freudenberger, D. Cordova, L. Flexner, et al., "Rynaxypyr™: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator," *Bioorganic & Medicinal Chemistry Letters*, vol. 17, 2007, pp. 6274-6279.
- [7] D. Cordova, E. Benner, M. Sacher, J. Rauh, J. Sopa, G. Lahm, et al., "Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation," *Pesticide Biochemistry and Physiology*, vol. 84, 2006, pp. 196-214.
- [8] U. Ebbinghaus-Kintscher, P. Luemmen, N. Lobitz, T. Schulte, C. Funke, R. Fischer, et al., "Phthalic acid diamides activate ryanodine-sensitive Ca²⁺ release channels in insects," *Cell calcium*, vol. 39, 2006, pp. 21-33.
- [9] D. B. Sattelle, D. Cordova, and T. R. Cheek, "Insect ryanodine receptors: molecular targets for novel pest control chemicals," *Invertebrate Neuroscience*, vol. 8, 2008, p. 107.
- [10] D. Cordova, E. A. Benner, M. D. Sacher, J. Rauh, J. S. Sopa, G. P. Lahm, et al., *Elucidation of the mode of action of Rynaxypyr™, a selective ryanodine receptor activator: Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2007.*
- [11] A. K. Isaacs, S. Qi, R. Sarpong, and J. E. Casida, "Insect ryanodine receptor: distinct but coupled insecticide binding sites for [N-C³H₃] chlorantraniliprole, flubendiamide, and [3H] ryanodine," *Chemical research in toxicology*, vol. 25, 2012, pp. 1571-1573.
- [12] A. Charnley and S. Collins, "10 Entomopathogenic Fungi and Their Role in Pest Control," *Environmental and microbial relationships*, vol. 4, 2007, p. 159.
- [13] R. Anand and B. N. Tiwary, "Pathogenicity of entomopathogenic fungi to eggs and larvae of *Spodoptera litura*, the common cutworm," *Biocontrol Science and Technology*, vol. 19, 2009, pp. 919-929.
- [14] G. Gupta, S. Rani, A. Birah, and M. Raghuraman, "Improved artificial diet for mass rearing of the tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae)," *International Journal of Tropical Insect Science*, vol. 25, 2005, pp. 55-58.
- [15] X.-M. Ma, X.-X. Liu, X. Ning, B. Zhang, F. Han, X.-M. Guan, et al., "Effects of *Bacillus thuringiensis* toxin Cry1Ac and *Beauveria bassiana* on Asiatic corn borer (Lepidoptera: Crambidae)," *Journal of invertebrate pathology*, vol. 99, 2008, pp. 123-128.
- [16] M. Ahmad, A. H. Sayyed, M. A. Saleem, and M. Ahmad, "Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan," *Crop Protection*, vol. 27, 2008, pp. 1367-1372.
- [17] T. Tefera and K. Pringle, "Effect of exposure method to *Beauveria bassiana* and conidia concentration on mortality, mycosis, and sporulation in cadavers of *Chilo partellus* (Lepidoptera: Pyralidae)," *Journal of invertebrate pathology*, vol. 84, 2003, pp. 90-95.
- [18] W. Abbott, "A method of computing the effectiveness of an insecticide," *J. econ. Entomol*, vol. 18, 1925, pp. 265-267.
- [19] P. Ferron, "Biological control of insect pests by entomogenous fungi," *Annual review of entomology*, vol. 23, 1978, pp. 409-442.
- [20] T. Anderson, A. Hajek, D. Roberts, H. Preisler, and J. Robertson, "Colorado potato beetle (Coleoptera: Chrysomelidae): effects of combinations of *Beauveria bassiana* with insecticides," *Journal of Economic Entomology*, vol. 82, 1989, pp. 83-89.
- [21] A. Hassan and A. Charnley, "Ultrastructural study of the penetration by *Metarhizium anisopliae* through dimilin-affected cuticle of *Manduca sexta*," *Journal of Invertebrate Pathology*, vol. 54, 1989, pp. 117-124.
- [22] A. Hassan, R. Dillon, and A. Charnley, "Influence of accelerated germination of conidia on the pathogenicity of *Metarhizium anisopliae* for *Manduca sexta*," *Journal of Invertebrate Pathology*, vol. 54, 1989, pp. 277-279.
- [23] G. D. Inglis, M. S. Goettel, T. M. Butt, and H. Strasser, "Use of hyphomycetous fungi for managing insect pests," *Fungi as biocontrol agents*, 2001, pp. 23-69.
- [24] V. D. Prasad, S. Jayaraj, and R. Rabindra, "Susceptibility of tobacco caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera) to certain entomogenous fungi," *Journal of Biological Control*, vol. 3, 1989, pp. 53-55.
- [25] H. Lin, X. Yang, Y. Gao, and S. Li, "Pathogenicity of several fungal species on *Spodoptera litura*," *Ying yong sheng tai xue bao = The journal of applied ecology/Zhongguo sheng tai xue hui, Zhongguo ke xue yuan Shenyang ying yong sheng tai yan jiu suo zhu ban*, vol. 18, 2007, pp. 937-940.
- [26] J. Purwar and G. Sachan, "Synergistic effect of entomogenous fungi on some insecticides against Bihar hairy caterpillar *Spilarctia obliqua* (Lepidoptera: Arctiidae)," *Microbiological research*, vol. 161, 2006, pp. 38-42.
- [27] A. Morales-Rodriguez and D. C. Peck, "Synergies between biological and neonicotinoid insecticides for the curative control of the white grubs *Amphimallon majale* and *Popillia japonica*," *Biological control*, vol. 51, 2009, pp. 169-180.
- [28] P. Duraimurugan and A. Regupathy, "Synthetic Pyrethroid Resistance in Field Strains of *Helicoverpa armigera* (Hubner)," *American Journal of Applied Sciences*, vol. 2, 2005, pp. 1146-1149.
- [29] S. Vijayavani, K. Reddy, and G. Murthy, "Pathogenicity of *Beauveria bassiana* (Deuteromycotina: Euteromycotina: Hyphomycetes) strains on *Spodoptera litura* (Fab.)," *Journal of Biopesticides*, vol. 2, 2009, pp. 205-207.
- [30] W. A. Al-Kherb, "Biological Characteristics of Orius albidipennis (Hemiptera: Anthocoridae) Reared on Insect and Mite Preys," *International Journal of Agricultural Research*, vol. 9, 2014, pp. 110-118.
- [31] R. Anand, B. Prasad, and B. N. Tiwary, "Relative susceptibility of *Spodoptera litura* pupae to selected entomopathogenic fungi," *BioControl*, vol. 54, 2009, p. 85.
- [32] J. C. Legaspi, T. Poprawski, and B. Legaspi, "Laboratory and field evaluation of *Beauveria bassiana* against sugarcane stalkborers (Lepidoptera: Pyralidae) in the Lower Rio Grande Valley of Texas," *Journal of Economic Entomology*, vol. 93, 2000, pp. 54-59.
- [33] A. K. Pandey and K. Kanaujia, "Effect of larval extract medium on pathogenicity of *Metarhizium anisopliae* (Metschnikoff) Sorokin against tobacco caterpillar, *Spodoptera litura* Fab.," *Journal of Entomological Research*, vol. 27, 2003, pp. 175-180.
- [34] J. Purwar and G. Sachan, "Biotoxicity of *Beauveria bassiana* and *Metarhizium anisopliae* against *Spodoptera litura* and *Spilarctia obliqua*," *Annals of Plant Protection Sciences*, vol. 13, 2005, pp. 360-364.
- [35] M. Amer, T. El-Sayed, H. Bakheit, S. Moustafa, and Y. A. El-Sayed, "Pathogenicity and genetic variability of five entomopathogenic fungi against *Spodoptera littoralis*," *Res J Agric Biol Sci*, vol. 4, 2008, pp. 354-367.
- [36] A. R. M. El-Geddawy, M. A. Ahmed, and S. H. Mohamed, "Toxicological evaluation of selected biopesticides and one essential oil in comparison with indoxacarb pesticide on cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: noctuidae) under laboratory conditions," *American-Eurasian Journal of Sustainable Agriculture*, 2014, pp. 58-65.
- [37] A. Tukaram, A. Hosamani, R. Naveena, and G. Santoshagowda, "Bioassay of flubendiamide on *Spodoptera litura* (Fab) population collected from different host crops," *Inter. J. Sci. Environ. Technol*, vol. 3, 2014, pp. 2225-2230.
- [38] S. M. Zaka, N. Abbas, S. A. Shad, and R. M. Shah, "Effect of emamectin benzoate on life history traits and relative fitness of *Spodoptera litura* (Lepidoptera: Noctuidae)," *Phytoparasitica*, vol. 42, 2014, pp. 493-501.
- [39] S. S. Bhatti, M. Ahmad, K. Yousaf, and M. Naeem, "Pyrethroids and new chemistry insecticides mixtures against *Spodoptera litura* (Noctuidae: Lepidoptera) under laboratory conditions," *Asian, J Agric Biol*, vol. 1, 2013, pp. 45-50.
- [40] E. Korrat, A. Abdelmonem, A. Helalia, and H. Khalifa, "Toxicological study of some conventional and nonconventional insecticides and their mixtures against cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)," *Annals of Agricultural Sciences*, vol. 57, 2012, pp. 145-152.
- [41] E.-S. A. El-Sheikh, "Comparative toxicity and sublethal effects of emamectin benzoate, lufenuron and spinosad on *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae)," *Crop Protection*, vol. 67, 2015, pp. 228-234.

- [42] M. M. Challa and S. K. Sanivada, "Compatibility of *Beauveria bassiana* (Bals.) Vuill. isolates with selected insecticides and fungicides at agriculture spray tank dose," *Innovare J Agric Sci*, vol. 2, 2014, pp. 7-10.
- [43] X. Liu, Q. Zhang, J. Z. Zhao, Q. Cai, H. Xu, and J. Li, "Effects of the Cry1Ac toxin of *Bacillus thuringiensis* on *Microplitis mediator*, a parasitoid of the cotton bollworm, *Helicoverpa armigera*," *Entomologia Experimentalis et Applicata*, vol. 114, 2005, pp. 205-213.
- [44] X.-x. Liu, Q.-w. Zhang, J.-Z. Zhao, J.-c. Li, B.-l. Xu, and X.-m. Ma, "Effects of Bt transgenic cotton lines on the cotton bollworm parasitoid *Microplitis mediator* in the laboratory," *Biological control*, vol. 35, 2005, pp. 134-141.
- [45] D. W. Roberts and R. A. Humber, "Entomogenous fungi," *Biology of conidial fungi*, vol. 2, 1981, p. e236.
- [46] T. Riasat, W. Wakil, M. Ashfaq, and S. T. Sahi, "Effect of *Beauveria bassiana* mixed with diatomaceous earth on mortality, mycosis and sporulation of *Rhizopertha dominica* on stored wheat," *Phytoparasitica*, vol. 39, 2011, pp. 325-331.

Table 4.1. Mean larval mortality (\pm SE) of 2nd instar larvae of *S. litura* exposed to *B. bassiana* (1.3×10^6) alone and in combination with Flubendiamide (24ppm), Chortrainiliprole (25 ppm) and Emamectin benzoate (9.5ppm) at different intervals

Treatment	Larval mortality (%) 2 nd instar					Expected mortality (%)	Co-toxicity factor	Type of interaction
	Day 2	Day 4	Day 6	Day 8	Day 10			
Bb	9.79 \pm 1.57d	18.94 \pm 2.52d	32.60 \pm 2.41d	40.37 \pm 1.68e	45.24 \pm 2.14e			
Flu	26.93 \pm 2.42c	43.01 \pm 1.72c	50.58 \pm 1.98c	56.40 \pm 2.15d	58.25 \pm 1.92de			
Chlor	31.48 \pm 1.79bc	49.05 \pm 1.73bc	54.39 \pm 2.14c	60.11 \pm 2.50cd	64.29 \pm 1.81cd			
Ema	40.47 \pm 2.35abc	54.76 \pm 2.24abc	61.53 \pm 1.43bc	66.56 \pm 2.05bcd	68.57 \pm 1.90cd			
Bb+Ema	42.01 \pm 2.18abc	56.77 \pm 2.33abc	65.13 \pm 1.04abc	71.69 \pm 1.86abc	74.60 \pm 2.04bc	113.81	-34.45	Ant
Bb+Flu	44.18 \pm 2.48ab	61.48 \pm 2.13ab	75.08 \pm 2.26ab	80.16 \pm 1.46ab	84.60 \pm 2.31ab	103.49	-18.19	Add
Bb+Chlor	8.68 \pm 2.25a	67.25 \pm 1.69a	79.42 \pm 1.97a	85.29 \pm 1.68a	91.43 \pm 1.57a	109.53	-16.53	Add

Table 4.2. Mean larval mortality (\pm SE) of 4th instar larvae of *S. litura* exposed to *B. bassiana* (1.3×10^6) alone and in combination with Flubendiamide (24 ppm), Chortrainiliprole (25ppm) and Emamectin benzoate (9.5 ppm) at different intervals

Treatment	Larval mortality (%) 4 th instar					Expected mortality (%)	Co-toxicity factor	Type of interaction
	Day 2	Day 4	Day 6	Day 8	Day 10			
Bb	6.67 \pm 1.57d	14.23 \pm 2.49d	26.30 \pm 1.54d	34.23 \pm 2.03d	40.58 \pm 2.28e			
Flu	24.71 \pm 1.56c	37.67 \pm 2.04c	45.98 \pm 2.35c	51.80 \pm 1.76c	53.76 \pm 2.13de			
Chlor	30.74 \pm 2.40bc	43.86 \pm 2.07bc	50.79 \pm 2.22bc	56.51 \pm 2.36bc	58.47 \pm 1.90cd			
Ema	35.13 \pm 2.34babc	50.79 \pm 1.81abc	56.82 \pm 2.32bc	61.16 \pm 1.90bc	62.33 \pm 1.88cd			
Bb+Ema	36.72 \pm 1.83abc	53.81 \pm 1.92ab	61.38 \pm 1.57abc	67.20 \pm 1.82ab	70.80 \pm 1.93bc	102.91	-31.20	Ant
Bb+Flu	40.53 \pm 1.81ab	57.14 \pm 2.22ab	65.82 \pm 1.81ab	71.59 \pm 2.42ab	76.88 \pm 2.34ab	94.34	-18.51	Add
Bb+Chlor	45.82 \pm 2.20a	62.33 \pm 2.41a	73.60 \pm 1.94a	80.21 \pm 1.78a	86.14 \pm 1.60a	99.05	-13.03	Add

Table 4.3. Mean developmental period (\pm SE) of *Spodoptera litura* exposed to *B. bassiana* (1.3×10^6) alone and in combination with sub lethal dosage of Flubendiamide, Chortraniliprole and Emmamectin benzoate against different life stages of *S. litura*

Treatment	Larval development (Days)	Pupal development (Days)	Adult longevity (Days)
Bb	17.89 \pm 1.74e	9.22 \pm 1.44cd	9.78 \pm 1.38ab
Flu	19.56 \pm 2.04de	10.11 \pm 1.38bcd	9.22 \pm 1.56abc
Chlor	20.56 \pm 2.16cde	10.89 \pm 1.59bcd	8.56 \pm 1.65abcd
Ema	21.11 \pm 1.89bc	11.11 \pm 1.98abc	7.78 \pm 1.44bcde
Bb+Ema	21.89 \pm 1.74abc	11.89 \pm 1.68ab	7.33 \pm 1.32cde
Bb+Flu	23.22 \pm 2.14ab	12.56 \pm 2.22ab	6.67 \pm 1.41de
Bb+Chlor	24.78 \pm 2.43a	13.56 \pm 2.16a	5.67 \pm 1.86e
Control	16.89 \pm 1.67e	8.56 \pm 1.74d	10.33 \pm 1.32a

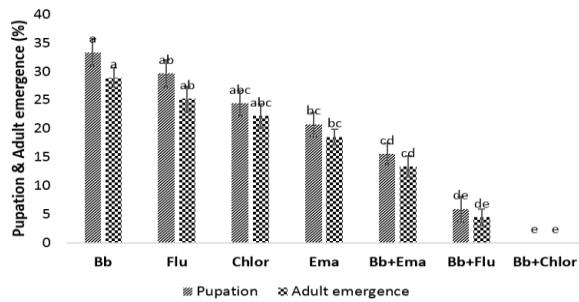


Fig. 4.1. Percent pupation and rate of adult emergence of 2nd instar larvae of *S. litura* exposed to *B. bassiana* (1.3×10^6) alone and in combination with Flubendiamide (24 ppm), Chortrainiliprole (25 ppm) and Emamectin benzoate (9.5 ppm).

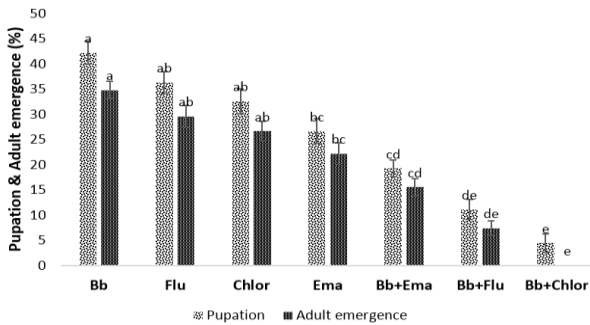


Fig. 4.2. Percent pupation and rate of adult emergence of 4th instar larvae of *S. litura* exposed to *B. bassiana* (1.3×10^6) alone and in combination with Flubendiamide (24 ppm), Chortrainiliprole (25 ppm) and Emamectin benzoate (9.5 ppm).

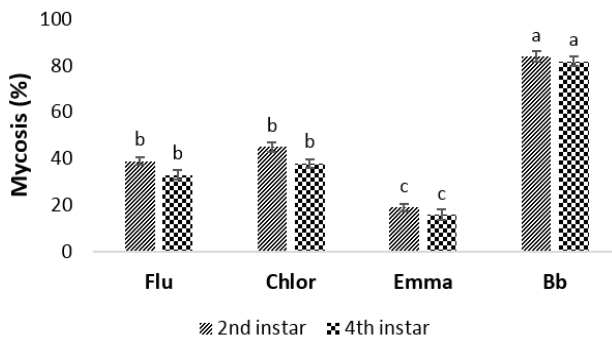


Fig. 4.3. Mycosis percentages (\pm SE) in cadavers of *S. litura* exposed to *B. bassiana* (1.3×10^6) alone and in combination with Flubendiamide (24 ppm), Chortrainiliprole (25 ppm) and Emamectin benzoate (9.5 ppm).

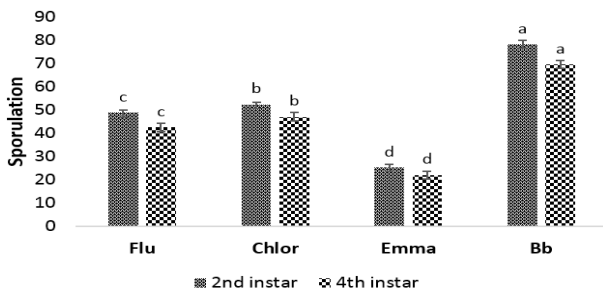


Fig. 4.4. Sporulation (conidia/ml) on cadavers of *S. litura* exposed to *B. bassiana* (1.3×10^6) alone and in combination with Flubendiamide (24 ppm), Chortrainiliprole (25 ppm) and Emamectin benzoate (9.5 ppm).

AUTHORS' PROFILES



Muhammad Ismail

belongs to Sargodha city, Pakistan. M.Sc (hons) Entomology (2013-2015), department of Entomology, University of Agriculture Faisalabad and B.Sc (hons) from University College of Agriculture, University of Sargodha. Currently in Zarai Taraqiati Bank Limited (ZTBL) Pakistan.

Two month's internship at Pest Warning & Quality Control of Pesticide in district Sargodha. (ismail.sgd.pak@gmail.com).



Waqas Wakil

Assistant Professor, Faculty of Agriculture, Department of Entomology, University of Agriculture Faisalabad. Research interest IPM of vegetable and stored grain insect pests. (arid1972@yahoo.com).



Nawaz Haider Bashir

from Pakistan. Ph.D Scholar having keen interest in biological control of pests at college of Plant Protection, Yunnan Agriculture University, China. M.Sc (hons) Entomology (2013-2015), Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad and B.Sc (hons) with major study Entomology (2009-2013) from University College of Agriculture, University of Sargodha. Three month internship at Pest Warning & Quality Control of Pesticide in district Layyah. (nawazuca@gmail.com).



Waheed ul Hassan

from Pakistan, and currently student of Master (Entomology) at Institut Pertanian Bogor, Indonesia. Bachelor degree in Agriculture at Sargodha University, Pakistan. Waheed ul hassan worked as field assistant for 6 month at Maize and Millet research Institute (MMRI) Sahiwal, Pakistan (waheedh20@gmail.com).



Usman Wali Muhammad

M.Sc (hons) Entomology (2013-2015), Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad and Graduated (2009-2013) from University College of Agriculture, University of Sargodha. He is attached Pesticide marketing in Pakistan. Three month's internship at Layyah Sugar Mill in Bio control Cell. (usmanuca@gmail.com).